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16. ABSTRACT

Bioremediation of creosote-contaminated materials is reviewed here by characterizing coal-tar creosote, identifying techniques for assessing the biodegradability of its many chemical constituents, examining known routes of microbial transformation of these chemicals, and reviewing the performance of previous bioremediation efforts. This approach is developed as a model system to project the potential application of bioremediation to ameliorate environments contaminated by complex mixtures of structurally diverse hazardous chemicals.

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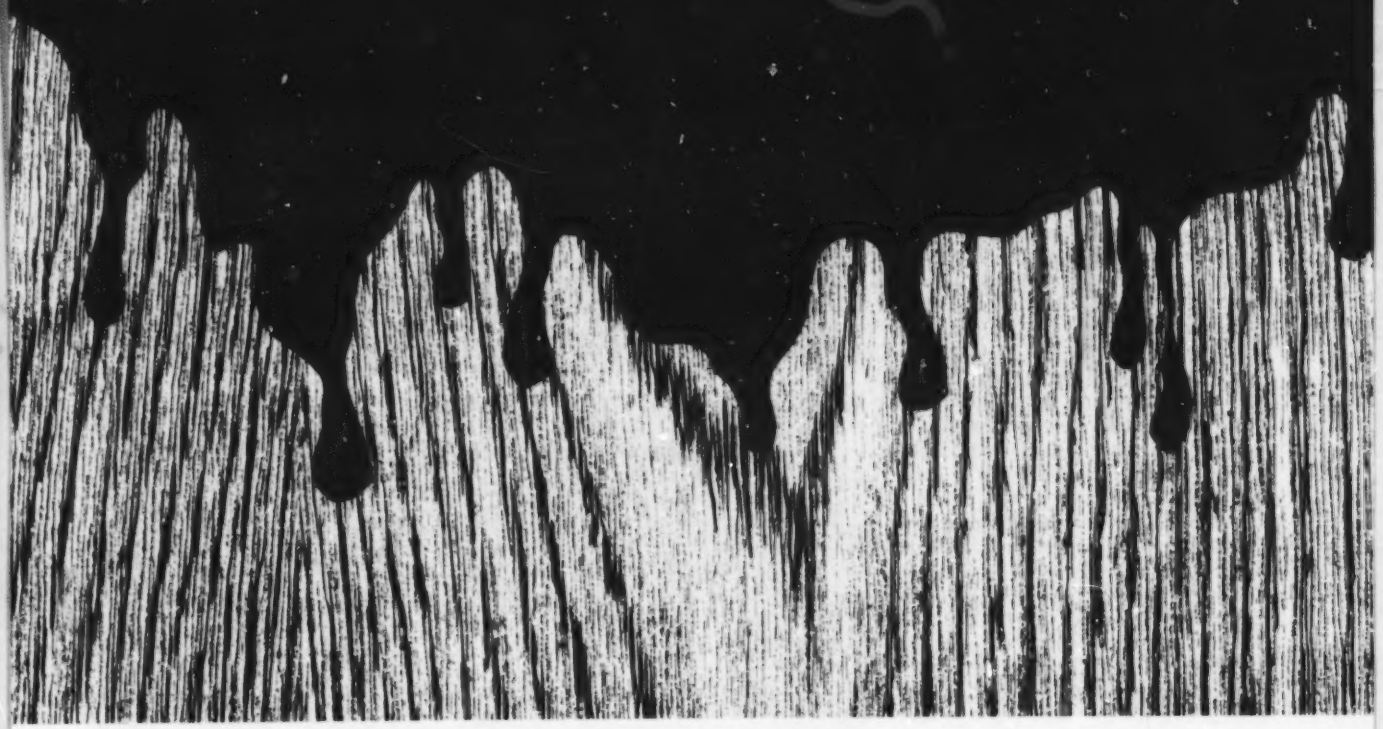

Creosote-contaminated sites

Their potential for bioremediation

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The wood-preserving industry uses more pesticides than any other industry worldwide. The major chemicals employed for this purpose are creosote, pentachlorophenol, and CCA (copper, chrome, and arsenate); hence their classification as the most widely used pesticides on a volume basis. Between 415 and 550 creosoting operations within the United States reportedly consume approximately 454,000 metric tons (4.5×10^7 kg) of creosote annually *(1)*.

When properly used and disposed of, creosote does not appear to significantly threaten human health. However, misuse, accidental spillage, and improper disposal of creosote results in contaminated environments with serious potential health risks. Bioremediation, the use of pollutant-degrading microorganisms to ameliorate contaminated materials, represents one means by which these sites may be restored to their original condition.

Environmental contamination

Creosote contamination is generally associated with surface soils, waters in treatment lagoons or evaporation areas, and groundwater contaminated with leachate from the above sources. Collectively, active wood treatment facilities generate an estimated 840 to 1530 dry metric tons annually *(2)* of hazardous-waste sludge classified as K001 (49 Code of Federal Regulations, Ch.1, subpart 172.101). Although the number of operating wood-preserving facilities has been reduced, it has been estimated that there are 700 sites throughout the United States where wood preservation is, or has been, conducted *(3)*. Because creosote treatment sites are commonly affected by leaking tanks, drippings from treated lumber, spills, and leachate from unlined holding ponds, this number presumably approaches the number of creosote-contaminated sites as well. A comprehensive analysis of all creosote-contaminated sites is not feasible; therefore, data generated from previous studies have considerable value in any evaluation of the extent and diversity of environmental creosote contamination. Further, these studies are useful for predicting the fate of creosote in con-

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taminated environments, and in developing appropriate responses.

Chemical composition of creosote

Creosote per se is a complex mixture of chemical constituents encompassing diverse chemical structures. Amelioration of creosote-contaminated sites therefore presents a challenge to technologies employing biological treatment of contaminated materials. Further, because of its mixed chemical nature, bioremediation of creosote provides an appropriate model for the treatment of sites similarly contaminated with mixtures of chemicals (e.g., petrogenic pollutant clean up, waste oil recovery, coal gasification).

Coal tar creosote is composed of approximately 85% polycyclic aromatic hydrocarbons (PAHs); 10% phenolic compounds; and 5% N-, S-, and O-heterocyclics. Of the approximately 150 to 200 chemicals in creosote, only a few are present in amounts of 1% or more. Thus, relatively few compounds typically represent 40–45% of a given creosote mixture.

In an effort to simplify the chemical composition of creosote to facilitate biodegradation studies, we have developed lists of the principal components in the PAH, phenolic, and heterocyclic fractions of coal tar creosote as shown in Tables 1, 2, and 3, respectively. Together, these fractions represent an estimated 95% of the total creosote and include some of the more toxic chemicals present in significant amounts. Because these compilations are based on field data obtained from both creosote-contaminated soil and water, they are presumed to approximate creosote as it would affect the native microflora in contaminated areas.

TABLE 1
Predominant polycyclic aromatic hydrocarbons in coal tar creosote

Compound	Relative percentage (wt)	MW	Aqueous solubility (mg/L, 25 °C)
Naphthalene	13	128.2	31.7
2-Methylnaphthalene	13	142.2	25.4
Phenanthrene	13	178.2	1.3
Anthracene	13	178.2	0.07
1-Methylnaphthalene	8	142.2	28.5
Biphenyl	8	154.2	7.5
Fluorene	8	166.2	2.0
2,3-Dimethylnaphthalene	4	156.2	3.0
2,6-Dimethylnaphthalene	4	156.2	2.0
Acenaphthene	4	154.2	3.9
Fluoranthene	4	202.3	0.26
Chrysene	2	228.2	0.002
Pyrene	2	202.3	0.14
Anthraquinone	1	208.2	—
2-Methylanthracene	1	192.3	0.04
2,3 Benzo[*b*]fluorene	1	216.3	0.002
Benzo[*a*]pyrene	1	252.3	0.003
Total = 17	100		

TABLE 2
Predominant phenolic compounds in coal tar creosote

Compound	Relative percentage (wt)	MW	Aqueous solubility (mg/L)
Phenol	20	94.1	82,000 (15 °C)
o-Cresol	10	108.1	25,920 (25 °C)
m-Cresol	10	108.1	23,500 (20 °C)
p-Cresol	10	108.1	24,000 (40 °C)
Pentachlorophenol	10	266.4	14 (20 °C)
2,5-Xylenol	7.5	122.2	3544 (25 °C)
3,5-Xylenol	7.5	122.2	4888 (25 °C)
2,3-Xylenol	5	122.2	4570 (25 °C)
2,4-Xylenol	5	122.2	6232 (25 °C)
2,6-Xylenol	5	122.2	6049 (25 °C)
3,4-Xylenol	5	122.2	4766 (25 °C)
2,3,5-Trimethylphenol	5	136.3	—
Total = 12	100		

Creosote degradation processes

Though coal tar creosote is generally considered to be environmentally recalcitrant, losses of creosote constituents occur with time. Hence, there are mechanisms for removing creosote from contaminated environments. Losses may occur through biotic processes and a variety of abiotic processes such as volatilization, leaching, and photodegradation *(4, 5)*. Because biodegradation represents the primary route through which creosote is removed from contaminated environments, exploitation of this naturally occurring process could yield an efficient means of remediating such sites.

For a compound to be degraded biologically under field conditions, several basic criteria must be met. First, an appropriate microbial community possessing the requisite catabolic ability must be present. As is the case with many other xenobiotics, the ability to degrade unique carbon sources is often associated with prior exposure of microbial communities to the chemical or to similar chemicals *(6)*. Second, bioavailability of the potential substrate must be considered along with the requirement for organism–substrate interaction. Lastly, environmental parameters such as temperature, redox potential, oxygen and nutrient availability, and moisture must be conducive to growth of the requisite organism(s).

The multiplicity of creosote's chemical components has prevented clear definition of creosote degradation. Overall, those studies that have attempted to describe the biodegradability of complex chemical mixtures such as creosote tend to be ambiguous and inconclusive. Conversely, microbial degradation of the individual components of creosote has been better described.

It is important to appreciate the limitations of laboratory studies of microbial degradation with a given compound present as the only available source of carbon and energy for microbial growth. Such studies may provide an inadequate indication of the degradation potential of this compound in the environment or in the presence of other organic compounds.

Thus, patterns of biodegradation of single compounds by pure cultures under laboratory conditions probably do not accurately reflect creosote biodegradation occurring in situ. Nevertheless, the information from such studies has proven useful in predicting the biodegradability of particular compounds under field conditions.

Biodegrading phenolic chemicals

Microbial degradation of phenolic compounds has been thoroughly reviewed *(7, 8)*. Several pentachlorophenol (PCP)-degrading bacteria have been isolated from PCP-contaminated soils and waters, and a variety of microorganisms—bacteria, yeasts, and fungi—have been isolated for their ability to degrade phenol and substituted phenols.

For example, Varga and Neujhar *(9)* isolated 13 different soil microorga-

TABLE 3
Predominant heterocyclic compounds in coal tar creosote

Compound (wt)	Relative percentage (mg/L)	MW	Aqueous solubility (mg/L)
N-Heterocyclics and *N*-containing aromatics			
Quinoline	10	129.2	6718 (20 °C)
Isoquinoline	10	129.2	4522 (20 °C)
Carbazole	10	167.2	1 (20 °C)
2,4-Dimethylpyridine	10	107.2	—
Acridine	5	179.2	5 (20 °C)
Aniline	5	93.1	3400 (25 °C)
2-Methylquinoline	5	143.2	—
4-Methylquinoline	5	143.2	—
Pyrrole	5	67.1	—
Pyrrolidine	5	71.2	—
S-Heterocyclics			
Benzo[*b*]thiophene	10	134.2	130 (20 °C)
Dibenzothiophene	10	184.3	2 (24 °C)
O-Heterocyclics			
Dibenzofuran	10	168.2	10 (25 °C)
Total = 13	100		

nisms with the ability to grow with phenol; six of these were bacteria, four were fungi, and three were yeasts. Roberts and Hedrick *(10)* isolated 10 phenol-degraders from a creosote waste pond. These organisms were classified as *Pseudomonas* and *Alcaligenes* species and were capable of degrading up to 45% of the total phenols (degradation of other creosote constituents was not assessed). These organisms can be readily obtained because of the widespread occurrence of phenolic compounds as natural products and as intermediates in the biodegradation of other biosynthetic compounds.

Growth of microorganisms at the expense of phenol and the methyl-phenol components of creosote will result in the proliferation of a diverse microbial population employing a variety of degradative pathways. Because of the convergent nature of pathways of aromatic metabolism, development of a large and diverse microbial population proficient in degrading phenolic compounds should furnish the requisite pathways for the later steps of degradation of PAHs and related compounds also present in creosote. Hence, mineralization of partially oxidized PAHs and heterocyclic chemicals may be greatly enhanced if cresol degradation is occurring simultaneously.

Biodegradation of PAHs

A number of reviews are available that outline enzymatic processes and describe microbial oxidation of polycyclic aromatic hydrocarbons *(11, 12)*. In the presence of appropriate organisms, biodegradation of lower molecular weight PAHs such as naphthalene is relatively rapid. Biological transformation of 3-ring PAHs such as acenaphthene, anthracene, and phenanthrene is usually slower. Organisms have also been studied that are capable of transforming higher molecular weight PAHs such as benz[*a*]anthracene *(13)*, benzo[*a*]pyrene *(14)*, fluoranthene *(15)*, and pyrene *(16, 17)*. In general, these compounds are usually co-metabolized and are not mineralized by pure microbial cultures.

A variety of microorganisms have been found to possess the ability to degrade PAHs: bacteria from the genera *Aeromonas, Alcaligenes, Beijerinkia, Cyanobacter, Flavobacterium, Micrococcus, Mycobacterium, Nocardia, Pseudomonas, Vibrio,* and the fungus *Phanerochaete chrysosporium*. In addition to pure-culture studies, biodegradation of creosote PAHs by mixed microbial communities has been demonstrated.

Biodegrading heterocyclic chemicals

Compared with the amount of data available describing the microbial degradation of phenolic and polycyclic aromatic compounds, relatively little is known about heterocyclic biotransformation processes *(18, 19)*. Generally, the aza-arene or nitrogen heterocyclic components of creosote are considered to be persistent and potentially hazardous to human health. However, under appropriate conditions, these compounds may be susceptible to microbial attack.

Biodegradation of O- and S-heterocyclic chemicals is poorly characterized, but initial reactions in the biotransformation of dibenzothiophene have been described and may represent a general mechanism for the biodegradation of these compounds *(19, 20)*. Though no known organism is capable of completely mineralizing dibenzothiophene, separate organisms have been found that are capable of mineralizing its biotransformation products.

For all creosote constituents, biotransformation under anoxic or anaerobic conditions is poorly understood *(21)*. Nonetheless, the existence of microorganisms potentially capable of degrading many creosote constituents suggests a potential for biologically mediated degradation of creosote by the indigenous microflora or inoculated organisms.

Bioremediation of sites

The basic principle of bioremediation is to exploit the ability of microorganisms to catabolize a wide range of organic substrates. Trickling filtration, land farming, activated sludge, oxidation lagoons, and soil inoculation represent a few means by which microorganisms are utilized to treat industrial wastes in situ. For bioreactor operations, engineering designs are based on the unique demands of a particular microbial consortium or pure culture so as to provide the ideal environment, thereby maximizing the process. When successful, bioremediation results in the conversion of a toxic chemical to nontoxic materials.

As might be expected, there are potential limitations which must be addressed if in situ bioremediation is to be successful. First, the pollutant in question must be in a chemical state conducive to microbial utilization. By and large, a chemical must be in an aqueous phase to enter a microbial cell and be biologically degraded (though some compounds may be transformed by extracellular enzymes). Typically, many recalcitrant compounds are only slightly soluble in water. Such hydrophobic pollutants tend to persist in nature, adsorbed to inert matrices. Application of surfactants or emulsifying agents to solubilize such compounds may accelerate the rate of microbial degradation.

Second, the addition of large amounts of organic carbon (as, for example, in the disposal of waste water from a wood-preserving facility) often results in the rapid consumption of oxygen and essential inorganic nutrients (nitrogen, phosphorus, sulfur, and potassium), thereby resulting in an anoxic and nutrient-limiting environment. Though a microbial population may exist with the potential to degrade the pollutant, the newly developed environment restricts its activity. Hence, aeration and nutrient supplementation are essential elements of many in situ treatments. Because biodegradation of the inorganic preservatives used in lumber treatment is not possible, secondary treatment for these compounds is required. This may involve reduction of hexavalent chromium to the trivalent form and its precipitation together with copper and arsenate.

Lastly, an essential aspect of success-

ful bioremediation entails the presence of an acclimated microbial population capable of degrading the pollutant in question. Because this is often a limiting factor, much effort has gone towards the isolation and characterization of degradative microbes. Theoretically, isolated organisms can be used to inoculate contaminated materials in which microbial communities capable of degrading the pollutant in question are nonexistent or scarce.

Case studies

Investigators hope to exploit the specificity and efficiency of microbially mediated processes to find biological systems potentially capable of ameliorating creosote- and other similarly contaminated sites; successful applications of in situ bioremediation of such contaminated soil and water have been reported *(1, 22)*. Lieberman and Caplan *(23)* reported the efficient removal of oil, grease, and PAHs by converting a creosote-waste impoundment lagoon into an in situ bioreactor. Within one week, 10-fold reductions in anthracene, benzo[*a*]pyrene, fluoranthene, and phenanthrene were observed.

Putilina *(24)* demonstrated that bacterial oxidation of phenolic wastes in effluent water from a coke production plant was both reliable and economical. Etzel and Kirsch *(25)* reported biological degradation of PCP in contaminated waste waters. Likewise, Rao *(26)* used trickling filters to remove phenols from waste generated by a wood-carbonization operation.

In many cases, the absence of acclimated microbial populations capable of degrading a given compound, or their presence in low numbers, is the major factor limiting rapid biodegradation of an environmental pollutant. Hence, Pignatello et al. *(27)* suggested that PCP-degrading organisms might be useful in remediating PCP-contaminated soils and waters. In studies by Edgehill and Finn *(28)*, direct inoculation of PCP-contaminated soils with 1×10^6 PCP-utilizing *Arthrobacter* (ATCC 33790) cells per gram of soil reduced the half-life of PCP from 2 weeks to < 1 day.

Subsequent studies by Crawford and Mohn *(29)* successfully remediated PCP-contaminated soils by inoculation with a PCP-degrading *Flavobacterium* (ATCC 39723). Using multiple inoculations, PCP concentration in a contaminated soil was reduced from 298 ppm to a final concentration of 58 ppm. However, the process required several months. Though inoculation with acclimated bacterial cultures accelerated the remediation process, manipulation of the soil environment (temperature, pH, O_2, water) also stimulated the native microflora to degrade PCP.

Often, however, simple inoculation of creosote-contaminated soils with creosote-degrading bacteria meets with limited success. Such failure may be due to a variety of reasons including die-off of lab-adapted strains, availability of substrate, and inability of inoculant organisms to compete with the native microflora. Therefore, successful restoration of creosote- and other similarly contaminated materials may require a proven microbial system and an appropriately contained process—one that optimizes microbial activity while buffering the system from toxic effects associated with a high degree of contamination.

With this in mind, various bioreactor designs have been engineered to house creosote-degrading organisms, providing them with a stable environment conducive to maximum creosote removal. Frick et al. *(30)* populated a specially designed bioreactor with a microbial consortium acclimated to creosote constituents and demonstrated the removal of 95% of the monitored PAHs from soil slurries and more than 99% removal of PCP from contaminated groundwater. Similar technology has been developed facilitating the removal of select PAHs (92–95% removal efficiency) from contaminated soils *(3)*.

Those chemicals that resisted biodegradation were not reported by Frick et al. *(30)*. However, higher molecular weight PAHs such as fluoranthene, pyrene, 2,3-benzo[*b*]fluorene, chrysene, and benzo[*a*]pyrene represent the type of compounds that tend to accumulate during bioremediation processes *(3)*. Hence, the development of microbial systems capable of transforming these compounds would enhance the effectiveness of biological systems for the treatment of creosote wastes. Natural adaptation processes may be accelerated via recombinant DNA technology to facilitate the realization of such microorganisms. Concomitant use of physical and chemical polishing steps may also serve to better the performance of bioremediation processes.

Examples of successful transfer of laboratory biodegradation studies to full industrial scale bioremediation processes can be cited. Pilot-plant and bench-scale studies by Dust and Thompson *(31)* employed activated-sludge, trickling-filter, and soil percolation processes to assess the efficacy of biological removal of pollutants produced by the wood-preserving industry. Soil percolation techniques removed more than 99% of the phenolic compounds (removal of other creosote constituents was not reported).

It was further demonstrated that 90% of PCP was biologically removed from waste effluent by using activated sludge inoculated with PCP-degraders previously isolated from a PCP-contaminated soil. Myers et al. *(32)* assessed the efficiency of various treatment processes at two independent wood-preserving facilities. The final waste waters at both sites were classified as "zero discharge," though select pollutants such as arsenic (20 μg/L) and PCP (105 to 633 μg/L) were present at trace levels.

Taking a slightly different approach, Blais *(33)* installed a pilot-plant fixed-film bioreactor at an abandoned wood treatment facility to degrade organic contaminants present in groundwater and storm water. Removal efficiency of creosote constituents ranged from 70% to 85%. Based on this information, on-site biological remediation was selected as the remedial action for treating creosote-contaminated soils and sludges at a former wood treatment operation in Minnesota *(34)*.

A full-scale treatment facility was constructed that resulted in 85% removal of creosote PAHs. Land-farming studies were performed at a former wood treatment plant in Montana to remediate 16,000 cubic yards of creosote-contaminated soil and sludge *(35)*. Though disappearance of creosote constituents was again evident, observed losses may have occurred through a variety of mechanisms including volatilization and photodegradation.

In summary, creosote-degrading organisms have been identified and have been successfully employed to remediate creosote-contaminated soils and waters. Nevertheless, to fully optimize the bioremediation potential of various creosote-degrading isolates, degradation patterns need to be better defined, systems conducive to maximum removal of the contaminants need to be developed, and microorganisms capable of mineralizing the more recalcitrant creosote constituents need to be isolated and characterized.

Further, the limited ability to biologically degrade complex chemical mixtures that are commonly found at hazardous waste sites needs to be expanded. The potential benefits of effective bioremediation technologies justify continued research to solve these problems and develop protocols for implementation. When fully developed, bioremediation will represent a reliable and efficient means of removing many organic pollutants from contaminated environments.

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